Evaluation of pH-sensitive poly(2-hydroxyethyl methacrylate-co-2-(diisopropylamino)ethyl methacrylate) copolymers as drug delivery systems for potential applications in ophthalmic therapies / ocular delivery of drugs

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ABSTRACT

Smart polymers like pH sensitive systems can improve different pharmacological treatment. In this work the behavior of copolymers containing 2-hydroxyethyl methacrylate (HEMA) with different proportions of 2-(diisopropylamino)ethyl methacrylate (DPA) and different amounts of cross-linker agent, ethylene glycol dimethacrylate (EGDMA) are evaluated as pH-sensitive drug delivery system for potential application in ophthalmic therapies. A detailed characterization of the pH-responsive behavior was performed by swelling studies and scanning electron microscopy (SEM) analysis. Drug loading and release studies at different pH values were evaluated using Rhodamine 6G (Rh6G) as a model drug. The interaction between Rh6G and hydrogels was studied by FTIR spectroscopy and SEM. The results show that the presence of DPA in the copolymers confers pH-responsive properties to the polymer, as noted in swelling and SEM studies, when the pH decreases below 7.40 the swelling degree increases and a porous morphology is observed. The apparent pK_a of copolymers was estimated between 6.80 and 7.17 depending on the composition. The amount of Rh6G loaded depends mainly on the medium pH and the interaction between the drug and the copolymers, observed by SEM and FTIR spectrum. The release of Rh6G of copolymers p(HEMA/DPA) show a normal Fickian or anomalous diffusion behavior at different pH values, depending on the HEMA/DPA ratio.

Keywords: Smart polymers; 2-hydroxyethyl methacrylate; 2-(diisoproylamino)ethyl methacrylate; pH-sensitive hydrogels; drug delivery systems.

1. Introduction

Hydrogels formed by chemical or physical crosslinking are a three dimensional structure made from hydrophilic polymer that can imbibe a considerable amount of water while maintaining their integrity. The use of hydrogels as drug delivery systems and biomedical devices has been extensive studied over the last few decades because of their biocompatibility properties and control of solute transport [1-3]. More recently, sensitive hydrogels, prepared with additional functions have gained considerable attention. In this way, the incorporation of stimuli-sensitive monomers in the chain of the hydrogel can improve the performance of the materials by increasing responsiveness in a particular medium [4]. These hydrogels, often called 'intelligent' or 'smart' hydrogels, can undergo relatively large and abrupt, physical or chemical modification in response to changes in the environmental conditions such as pH, ionic strength, temperature or in presence of specific chemical compounds [5, 6]. For this reason, they are usually known as environmentally sensitive hydrogels. These types of stimuli-responsive polymers have the property to swell, shrink, bend, or degrade in response to external changes in the environmental conditions.

Due to its properties, sensitive hydrogels have been proposed for a number of applications like drug delivery, separation techniques and sensors [7-9]. Recently sensitive hydrogels have been also proposed for ocular drug delivery systems in order to improve the ocular bioavailability of drugs, and to reduce the appearance of side effects [10]. In this case, within topical application of drugs, the presence of ocular compact barrier in the corneal and conjunctival epithelia of the eye, along with the dynamics of the lacrimal system, hinder the drug absorption into the intraocular area [11, 12]. The use of sensitive polymeric hydrogels allows to extend the resident time of drugs in the eye and increased the amount of absorbed drug [13].

In recent years research were mainly focused in the technological innovations in this field with the aim to design hydrogels for an specific use as ocular drug delivery systems or in order to improve the uptake capacity and the release performance of these systems [8, 11, 14] although few works have been published using intelligent systems.

Of particular interest in this work it is the use of smart polymers as ocular drug delivery systems, and specifically the use of pH-sensitive hydrogels. This kind of material has been extensively studied as drug delivery systems for different applications, mainly due that they

can release the drug selectively according to the pH of the medium. In the human body there are variations in the physiologic pH values in both normal and pathological conditions, for example the gastrointestinal tract presents heterogeneous environments with different pH values ranging from 1 to 7.5 [15]. These conditions allow pH-sensitive hydrogels to release the desire drug in the right place. In case of ophthalmologic therapies the release should be close to the medium ocular pH of 7.45 [16, 17].

The potential use of stimuli-sensitive hydrogels allow not only a spatial control but also a temporal control; i.e. during the period of time when the pH value is outside the normal range. These pH-sensitive hydrogels as drug delivery systems are potentially useful in ophthalmic therapies due that deviations from normal pH were observed in some disease processes, for example in ocular rosacea (an inflammation of the eye) [18]. Ocular pH changes also in allergy and other conditions such as dry eye and bacterial infections [19]. Therefore these systems may be useful for controlling drug release in response to the pathological conditions.

Moreover, pH-sensitive hydrogels are normally prepared by adding pendant acidic or basic functional groups to the polymer backbone by including during the polymerization monomers like N-(3-aminopropyl)methacrylamide, 2-(dimethylamino)ethyl methacrylate, methacrylic acid and 2-aminoethyl methacrylate and 2-hidroxyethyl methacrylate (HEMA) for ionic type and butyl methacrylate, allyl diglycol carbonate, diallyl phthalate, and methyl methacrylate as neutral hydrophobic types [20-24]. Recently, we have reported the synthesis of a new pH-sensitive hydrogel based on 2-(diisopropylamino)ethyl methacrylate (DPA) and 2-hidroxyethyl methacrylate (HEMA), p(HEMA-co-DPA) [25] with good film properties depending on the monomers ratio.

In this contribution, we evaluate the properties of these pH-sensitive hydrogels with different proportions of HEMA and DPA and two degrees of cross-linking as potential materials for using in ocular drug delivery systems. Owing to this material can undergo physical or chemical modifications in response to changes in the environmental conditions, it is necessary to characterize the morphology and swelling behavior in function of the pH to understand and predict the drug's release rate. In this work we studied the swelling behavior of the hydrogels in a range of pH from 5.5 to 8.4 which is the used range in eye drop solutions, and at the average ocular temperature of 34.5 ± 0.5 °C [26, 27]. We have determined the pK_a values of different copolymers' compositions, and also we have studied morphological changes on hydrated samples at different medium pHs using Scanning Electron Microscopy (SEM). Their potential for ophthalmological application as pH-sensitive control drug release system, were investigated in vitro using Rhodamine 6G Chloride (Rh6G, M_w = 479.01) as a model drug,

because it is stable in water solutions (water solubility at 25 °C = 20 mg L^{-1}) and it is easily detected by its UV absorption. The effects of HEMA/DPA ratio and different cross-linking degrees on drug uptake and release behavior were studied at different pHs value at ocular conditions. Despite the number of recent studies incorporating active agent into hydrogels, the interaction between the matrix and the drug receive limited attention in the literature and it is often overlooked [4]. That is why in this work we intended to analyze also the hydrogels performance and the interactions with the model drug by using FTIR and SEM analysis.

2. Experimental section

2.1. Materials

2-hydroxyethyl methacrylate (HEMA, 97 %) and the cross-linker, ethylene glycol dimethacrylate (EGDMA, 98 %), were purchased from Sigma-Aldrich, while 2-(diisopropylamino)ethyl methacrylate (DPA) were purchased from Scientific Polymers Products. Darocur TPO (97 %) from Sigma-Aldrich was used as the initiator. The phosphate buffer solutions (PBS) were prepared from standard chemicals. The study of the hydrogels as drug delivery systems was performed using Rhodamine 6G Chloride (Rh6G, 95 %), from Sigma-Aldrich, as model drug (Figure 1). This molecule is frequently used for those studies because it has a similar chemical structure compared to drugs [28]. Additionally Rh6G have good solubility in water (20 g/l at 25 °C), chemical stability and it is easy detectable with UV-visible spectroscopy.

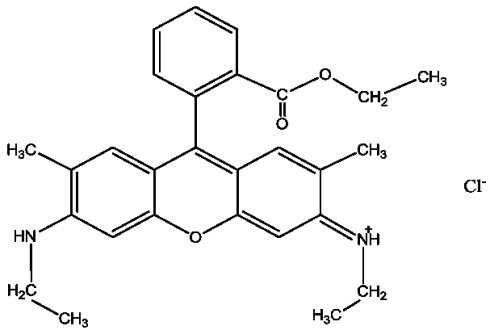


Figure 1. Chemical structure of Rhodamine 6G cation.

2.2. Polymer synthesis

The synthesis of DPA homopolymer and copolymers was performed in bulk by free radical polymerization using diphenyl (2,4,6-trimethylbenzoyl)-phosphine oxide (Darocur® TPO) as

photo-initiator. Different ratios of HEMA/DPA monomers (namely 100/0, 90/10 and 70/30) and 1 and 3 wt. % of cross-linker (relative to the whole monomer) were mixed with 1 % w/v of photo-initiator and irradiated with an UV-lamp (Rayonet RPR3500). More details of the synthesis and experimental procedures can be found in a previous paper [25]. The films with 180 ± 30 μm of thickness were cut into circular pieces of 13 mm of diameter with a cork borer and dried at 25.0 ºC for 48 h before use. Film samples were denoted by using a short-hand notation HDX/Y-n, where X and Y denote the HEMA (H) and DPA (D) content respectively, and n denote the amount of cross-linker.

2.3. Swelling degree

For the determination of the swelling degree, dry samples were immersed in phosphate buffer solutions (PBS, 0.1 M) at the desired pH (ranging from 5.5 to 8.4) at the average ocular temperature of 34.5 °C [27]**.** At regular periods of time the samples were removed from the aqueous solution, blotted with filter paper to remove surface liquid, weighed and returned to the same container until reaching a constant weight. The equilibrium swelling degree (Q_e) was calculated using the following equation:

$$Q_e(\%) = \frac{(W_e - W_d)}{W_d} * 100 \quad (1)$$

where W_d is the weight of the dry film and W_e is the weight of swollen film at equilibrium. The experiments were performed in triplicate.

2.4. Drug loading

The films were loaded with Rh6G by soaking the dry films into 20 ml of the drug solution (50 mg/L) in PBS at pH 6.5 and 8.4, and at 25.0 °C, until the equilibrium was reached. The drug uptake kinetics were followed by measuring the absorbance of the solution by UV-visible spectroscopy at 348 nm using a Fluorat®-02-Panorama spectrophotometer, Lumex, Russia. Samples loaded with Rh6G were denoted by adding the letter R in brackets to the name of the sample (e.g. HD90/10-1(R)).

2.5. Scanning Electron Microscopy (SEM)

The morphology of the hydrogels and the drug distribution were observed by Scanning Electron Microscopy (SEM) with an FEI - Quanta 200 (The Netherlands) instrument, in high vacuum mode and operated at 15 or 20 kV acceleration voltage. The p(HEMA-co-DPA) and

pHEMA hydrogels were equilibrated during 24 h in different buffer solutions and then were frozen at – 40 °C in an alcoholic solution followed lyophilization under vacuum for 24 h. In order to prevent sample-charging effects during the observation, fractured pieces of the samples were mounted onto the surface of an aluminum SEM specimen holder and sputter-coated with a thin overlayer of gold before observation. Films loaded with Rh6G were prepared in the same way.

2.6. Infrared spectroscopy (FTIR)

The FTIR spectra were measured in transmission mode using a FTIR Nicolet 380 spectrometer, Thermo Scientific, USA. Samples were loaded with Rh6G until the equilibrium was reached and then powdered and mixed with KBr; disks were formed by pressing. The FTIR spectra were obtained by recording 64 scans between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}. Spectra processing was performed using the software EZ Omnic.

2.7. Drug release experiments

The release experiments of Rh6G loaded p(HEMA-co-DPA) films were conducted in different pHs mediums. The drug loaded films were removed from the loading solution, wiped with filter paper to remove surface liquid and placed directly into the release solution. Drug release experiments were performed by immersing the films into 20 mL of PBS (0.1 M) at 34.5 °C. The dynamic drug concentration in the PBS solution was monitored by measuring the absorbance at 526 nm. The Rh6G concentration released as a function of time (t) was adjusted to a power-law type relationship [29, 30] using the equation of Ritger-Peppas:

$$\frac{M_t}{M_e} = kt^n \tag{2}$$

Here M_t and M_e are the cumulative amount of drug released after a time t and at infinite time, respectively, while k is a constant related to kinetic behavior and experimental conditions, and n is the release exponent depending on the release process. The data were fitted only up to 60 % of drug release in order to apply equation 2.

The parameters k and n were calculated from the intercept and the slope of the following equation:

$$ln(M_t / M_e) = ln\, k + n\, ln\, t \tag{3}$$

For Fickian diffusion processes, the following equation applies to calculate the diffusion coefficient (D_{ip}), where L is the thickness of the film:

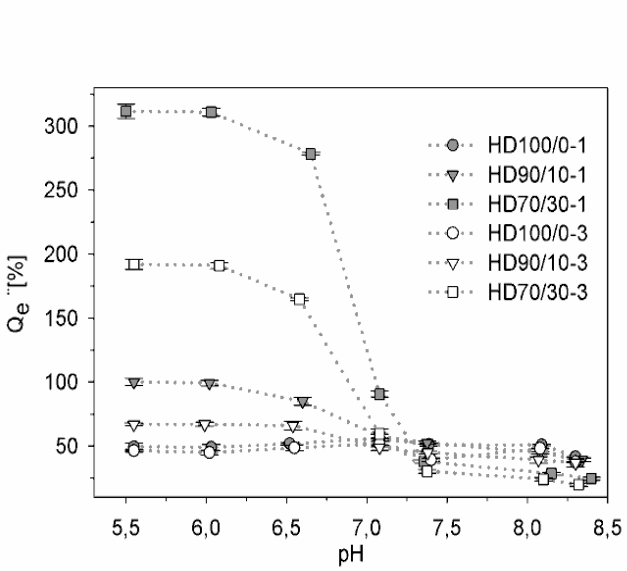
$$\frac{M_t}{M_e} = 4\left(\frac{D_{ip}t}{\pi L^2}\right)^{1/2} \quad (4)$$

189

190 **3. Results and discussion**

191 **3.1. Swelling studies**

192 In pH-sensitive systems the release rate of the drug is regulated by several factors as swelling
193 degree, drug-matrix interaction, water content and the initial active principle (PA)
194 concentration [31, 32]. However the swelling behavior as a function of pH has a principal role
195 in drug release regulation, which makes this technique to be an important tool to predict the
196 release rate of the drug. In this section are presenting the swelling results of the hydrogels in a
197 range of pH from 5.5 to 8.4 and the determination of pK_a values corresponding to the different
198 compositions of the copolymers. Figure 2 shows the equilibrium swelling degree for different
199 HEMA/DPA ratios with 1 and 3 wt. % of cross-linker at different pH values.



200

201 **Figure 2**. Equilibrium swelling degree for copolymers with different HEMA/DPA ratios, with
202 1 and 3 wt. % of cross-linker as a function of pH at 34.5 °C.

203

204 Hydrogels of pHEMA show a slight increase in the swelling degree when increasing the pH
205 from 5.5 to 8.4. However, the difference of swelling degree over the pH range studied in this
206 work is rather low. Similar results were observed by Brannon-Peppas and Peppas [33]. On the
207 other hand, hydrogels of p(HEMA-co-DPA) show a significant increase of the swelling when
208 the pH decrease below 7.40. This effect is directly proportional to the amount of DPA co-
209 monomer present in the copolymers, and is mainly attributed to the protonation of the tertiary

amino groups. At pH below 7.40, amino groups become protonated and the electrostatic repulsion, between these ionized groups, expand the network space and increases its internal volume, allowing water to get into the matrix. [14, 34, 35]. The equilibrium of swelling is reached around pH 6.0. At basic pH, above 7.40, the effect is reversed, the swelling degree decreases with the amount of DPA present in the hydrogel. In this case functional groups of HEMA and DPA are able to form hydrogen-bonds [25], which in turn generate a proximity between the polymer chains and consequently reduce the free space available for water molecules. Additionally, swelling values decrease also due to the hydrophobic nature of the unprotonated DPA moiety at basic pH.

For all HEMA/DPA ratios the cross-linking density does not modify the sensitivity to respond to pH changes but it affects the swelling degree. For high proportion of cross-linking, the swelling decreases for a given pH. This behavior is due mainly to two factors: first a matrix with higher crosslinking density has less free space to be occupied by water; and second the crosslinking degree generates a more rigid tridimensional structure which limits the mobility of the chains and increases the elastic force that opposes to the expansion of the internal space of the hydrogel [36].

The apparent pK_a of copolymers can be estimate by using swelling experiments at different pHs. In the pH range of 6.5 to 7.4 the swelling of the hydrogel decreases almost linear when increasing pH. In this range of pH, both the protonated and unprotonated form of the DPA moiety are present inside the polymer matrix acting as a buffer system in the hydrogel. Under these conditions, the Handeson-Hasselbalch equation can be applied to determine the apparent pK_a:

$$pH = pK_a + \log\left[\frac{\text{unprotonated state of tertiary amine group}}{\text{protonated state of tertiary amine group}}\right] \quad (5)$$

The apparent pK_a of the hydrogel buffer system can be determined from the pH value for which the fraction between these two forms is one. This corresponds to the point located in the middle of the swelling curve presented in Figure 2. The apparent pK_a values are show in Table 1 and range from 6.80 to 7.17 depending on the composition and the crosslinking degree of the polymers, due to the availability of the ionic groups of the hydrogel to act as buffer system [29].

Table 1. Apparent pK_a values for copolymers with different HEMA/DPA ratios and with 1 or 3 % wt. of cross-linker at 34.5 °C.

Copolymers	*pK_a*	*SD*
HD70/30-1	7.01	0.06
HD70/30-3	6.94	0.07
HD90/10-1	6.87	0.08
HD90/10-3	6.80	0.06

3.2. Drug uptake

Figure 3 shows the cumulative uptake of Rh6G as a function of immersion time for pHEMA and p(HEMA-co-DPA) films in PBS at pH 6.5 (a) and 8.4 (b).

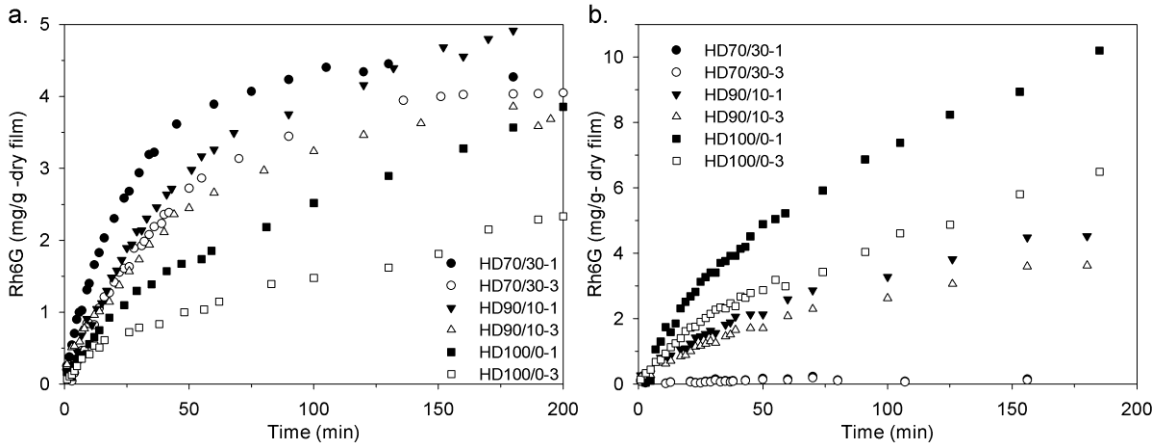


Figure 3. Cumulative uptake of Rh6G as a function of immersion time for pHEMA and p(HEMA-co-DPA) films in PBS at pH 6.5 (a) and 8.4 (b).

At pH 6.5 by increasing the DPA content, the Rh6G kinetic uptake increases (Figure 3a). At this pH the swelling degree of the hydrogels containing DPA (see above) favors the incoming of the drug into the film due to the increase of the network space and the diffusion of the aqueous solution, allowing the water soluble drug to get into the matrix. However, the final uptake at acid pH for pure pHEMA films is higher than that for the copolymer films (Table 2). This behavior can be explained in terms of an increased electrostatic repulsive interaction between the protonated tertiary amine groups of the polymer matrix and the positive charge on the Rh6G cation when increasing DPA content (see Figure 1 for the Rh6G chemical structure).

Table 2. Final mass uptake of Rh6G at pH 6.5 and 8.4.

HEMA/DPA	***Cross-linking (% wt.)***	***Rh6G uptake (mg/g of dry film)***			
		pH 6.5		***pH 8.4***	
		Mean	*SD*	*Mean*	*SD*
100/0	1	27.4	0.1	31.8	0.3
	3	24.6	2.0	31.4	0.9
90/10	1	6.3	0.6	23.7	0.8
	3	5.2	1.6	20.0	0.2
70/30	1	4.7	1.1	18.0	0.9
	3	2.7	0.9	15.1	0.2

SD: standard deviation; n: number of measure between 2 - 4

On the opposite, at pH 8.4, by increasing the DPA content, the Rh6G kinetic uptake decreases (Figure 3b), and it is slower than at acid pH. At this pH the increment on the DPA content causes a decrease in the swelling degree and consequently a decrease in the network space that retard the drug incorporation. However, the final uptake of Rh6G at pH 8.4 is higher than at pH 6.5. At acidic pH, the swelling increases due to the protonation of the functional group of the DPA, but the incorporation of Rh6G decreased by the electrostatic repulsion between the tertiary amine of DPA (partially protonated) and the cation of Rhodamine 6G. At basic pH, the electrostatic repulsion is less pronounced due to the decrease in the degree of ionization of the matrix, and hence the incorporation of Rh6G in the copolymers is higher. In conclusion, the amount of Rh6G incorporated into the polymer is inversely proportional with the swelling of the hydrogel, and depends mainly on the medium pH and the interaction between the drug and the matrix of the copolymers.

At both pH values the total uptake of Rh6G is higher for pure pHEMA homopolymer than for the copolymers. By including DPA monomer, the total amount of OH groups present in the hydrogel is reduced (as verified by FTIR) and, consequently, the available interaction sites decrease and then the number of Rh6G molecules incorporated also decreases (see Table 2).

As expected by the swelling data, increasing the degree of cross-linking from 1 to 3 wt. % reduces the amount of Rh6G incorporated in all cases. Thus it is possible to modify the final incorporation of Rh6G changing the pH of the loading medium instead of modifying the loading time. This allows regulating the amount of load drug into the hydrogel depending on the dose that is to be released.

3.3. SEM characterization

SEM is probably the best method for characterizing the hydrogel structure, especially in drug delivery systems because it offers information of surface porosity, amorphous and crystalline characterization, particle size, phase separation and in particular the active principle ingredient distribution in the structure [37]. Morphologic changes of lyophilized pH-responsive hydrogels, after exposure them to aqueous solutions of different pH values (6.5 and 8.4), have been examined by SEM technique and the images are shown in Figure 4.

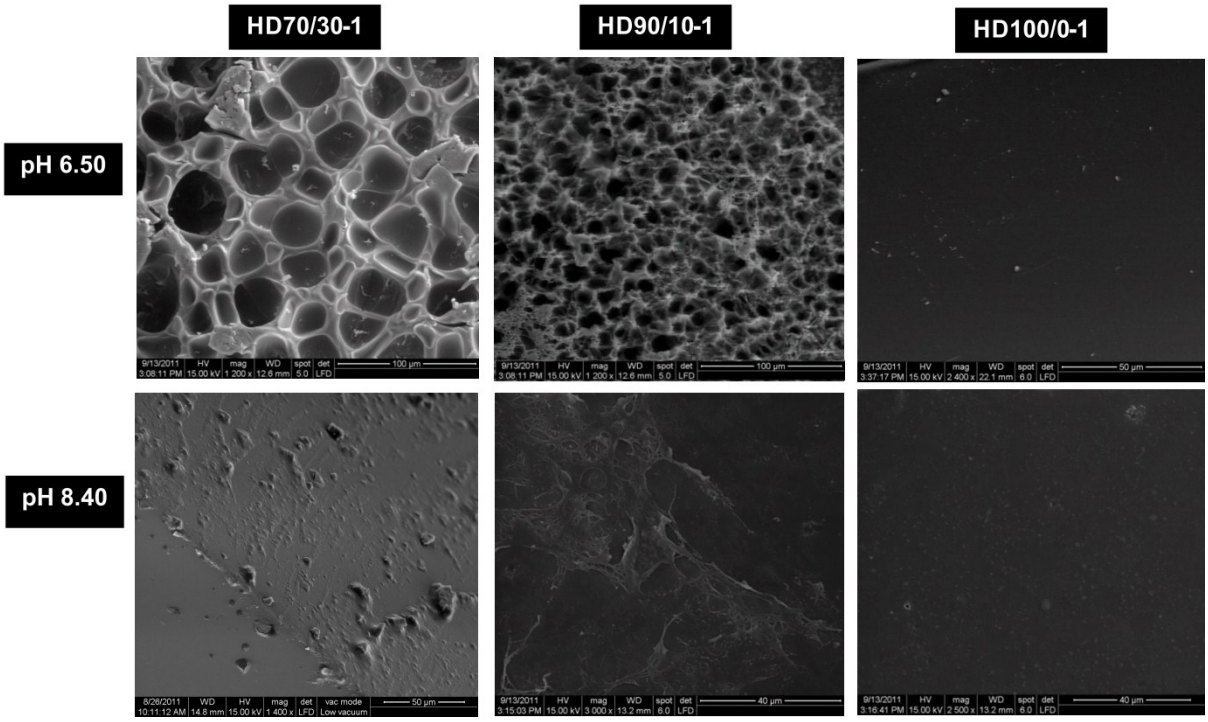


Figure 4. SEM images of the surface of hydrogel HD70/30-1, HD90/10-1 and HD100/0-1 at pH 6.5 and pH 8.4.

The surface of the hydrogel HD70/30-1 at pH 6.5 shows an open morphology state with a porous structure, thin walls and a predominant free space as a consequence of the matrix expansion at this pH. At pH to 8.40 a collapsed state is observed with almost a featureless structure due to lower swelling degree and a more hydrophobic polymer at this pH. For hydrogel HD90/10-1 the surface also shows a morphological change with the pH value. At pH 6.50 a homogeneous pore distribution on the surface is observed, while at pH 8.40 a non-porous and compact surface is appreciated. When the DPA content is higher, the equilibrium swelling increases, which led to more and highest pores in the hydrogels as obtained from lyophilization, being 5 ± 2 μm for 10 wt. % and 7 ± 2 μm for the 30 % of DPA. By comparing those values with the mesh sizes of conventional hydrogels, smaller than 100 nm [38, 39], both systems have a higher pore size. For hydrogel HD100/0-1 no changes with pH are

appreciated and in all cases a compact surface is observed. The incorporation of DPA confers pH-responsive properties to the polymer, as noted in swelling studies; therefore changing the medium pH not only changes the film volume but also the morphology. In the case of sample with higher concentration of cross-linker (3 wt. %) the same trend in morphological changes with the medium pH is observed (data not shown).

In Figure 5 are presented the SEM images of samples HD70/30-1(R), loaded with RhG6 at pH 6.5 and 8.4.

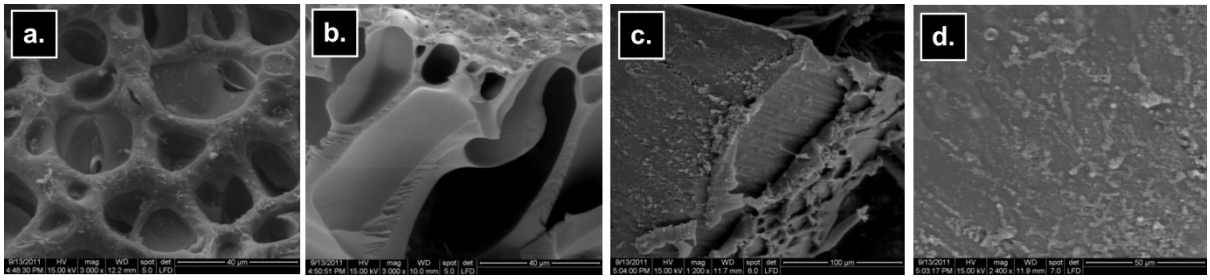


Figure 5. SEM images of hydrogel HD70/30-1(R) loaded at pH 6.5: a) inside the matrix, b) surface; and at pH 8.4: a) inside the matrix, b) surface.

The SEM images of the hydrogel 70/30-1(R) loaded at both pHs (6.5 and 8.4) show the presence of Rh6G. In the case of loading at pH 6.5, the surface (Figure 5.a) shows a greater accumulation of Rh6G unlike inside the matrix (Figure 5.b). While at pH 8.4 the Rh6G is observed both inside the matrix and on the surface (Figure 5c and 5d respectively). Rh6G molecules have lower affinity for the matrix of the copolymer HD70/30-1 at acidic pH, and therefore incorporation is lower and mainly superficial.

3.4. FTIR spectroscopy

Figure 6 shows the FTIR spectra of HD70/30-1 and HD100/0-1 with Rh6G (samples labeled R) and without Rh6G. The main differences in the high wavenumbers region of the spectra are the increasing intensity of the stretching band of the O-H group (3414 cm^{-1}). The existence of an interaction between Rh6G and -OH groups through the group =N+(H) is known, therefore is also expected interactions between Rh6G and pHEMA [40].

The C-H stretching region of spectra is also different. Upon the copolymerization with DPA, the $-CH_2-$ and $-CH_3$ bands of the stretching modes of pure pHEMA observed at 2986, 2951 and 2882 cm^{-1} (Figure 6) are overlapped with those of the DPA monomer and the peaks

become broader. In the C-H stretching region of the HD70/30-1 copolymer spectrum, a broad band centered at 2965 cm^{-1} is observed. This wavenumbers corresponds to the characteristic peak of the methine group in the isopropyl moiety ($(CH_3)_2$-CH-) [41]. However after Rh6G loading the peak pattern of the FTIR changed showing peaks at 2985, 2950 and 2888 cm^{-1}. These peaks values are close to the C-H stretching of the pure pHEMA, suggesting that the peak of the stretching vibration of the methine group at 2965 cm^{-1} shifted, probably to higher wavenumbers and overlapping with the 2985 cm^{-1} peak due to the loss of interaction between the lone electron pair of tertiary amine group of DPA with the OH of pHEMA [25] and the formation of new hydrogen bonds with the Rh6G molecules [40].

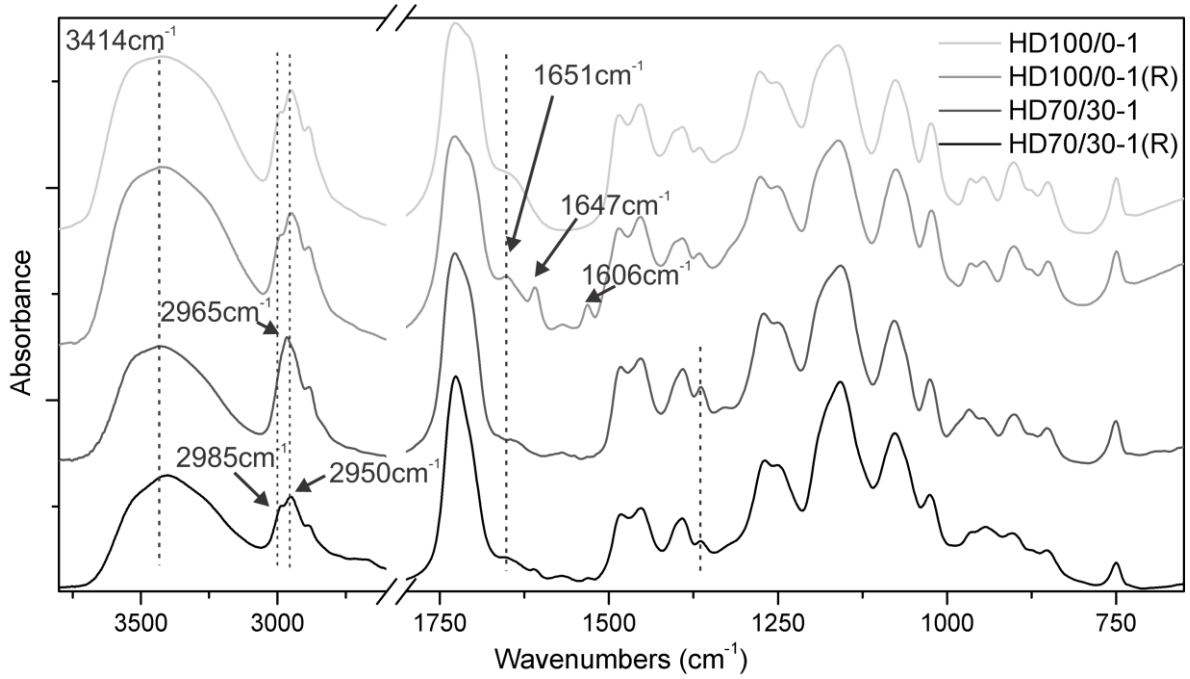


Figure 6. FTIR spectra for the films: HD100/0-1, HD100/0-1(R), HD70/30-1 y HD70/30-1(R).

In the 2000 – 400 cm^{-1} region the new features after Rh6G loading are mainly in the low wavenumbers side of the C=O (free) stretching band (located at 1730 cm^{-1}), namely a band at 1651 cm^{-1} from the contribution of bonded carbonyl groups of Rh6G, and small peaks at 1647 and 1606 cm^{-1} from the xanthene ring of this molecule (see [42, 43] for more details of Rh6G spectrum). Those contributions are more evident in HD100/0-1 film due to its higher loading of Rh6G. In HD70/30-1 film, the band of the isopropyl group [$(CH_3)_2$-CH-] of DPA moiety, observed at 1336 cm^{-1} in the unloading film, shows lower intensity after Rh6G loading. Minor differences are also observed in the 1000 – 880 cm^{-1} region, where the contribution of the

absorption bands of Rh6G is negligible. This region is associated to C-C modes of the carbon backbone of the polymer.

In summary, the observed differences in the FTIR spectra between loaded and unloaded films, indicate that the Rh6G molecules are interacting with the polymer chains, probably with both parts of the copolymer (HEMA and DPA moieties) by hydrogen bonding or through dipole–dipole interaction [44].

3.5. Drug release

3.4.1 Effect of medium pH

Figure 7 shows the cumulative concentration of Rh6G released at 34.5 °C in PBS for different pH values as a function of time for HD70/10- 1, HD90/10-1 and HD100/0-1 loaded at pH 8.4. This pH was chosen because the drug uptake is the highest found in this work (see section 3.2 and Table 2.)

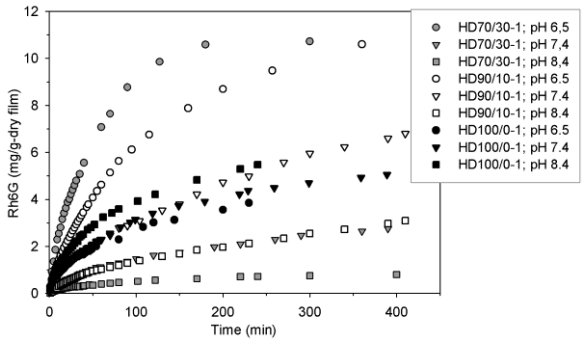


Figure 7. Cumulative concentration of Rh6G released as a function of time for HD70/10-1, HD90/10-1 and HD100/0-1 (loaded at pH 8.4) at 34.5 °C in PBS for different pH values.

In copolymers of HEMA/DPA release kinetics of Rh6G varies significantly with changing the pH of the medium, as the pH increases the release becomes faster. This effect is related to the swelling property of these hydrogels when changing the medium pH. At basic pH the matrix is closed and the swelling is low, by acidifying the medium, the hydrogel swells and the release rate of the Rh6G increases as well as the pore size of the hydrogel [45]. In the case of HD70/30-1 the effect of the pH on the release kinetics is much more pronounced than in HD90/10-1 copolymer due to the higher amount of DPA. At acid pH, the electrostatic

repulsion between the tertiary amine groups of the DPA partially protonated and the Rh6G cation favors the released of this drug.

Drug release behavior at different pH values is also consistent with the morphological characteristics observed in the SEM images (Figure 3), the higher the pore size of the hydrogel, the faster is the released of the drug.

The total amount of Rh6G released from pHEMA film is incomplete (about 30 % is released) and it is almost pH independent as a consequence of the interaction of the Rh6G molecule with the polymer functional groups, as observed by FTIR spectroscopy. Similar results were obtained in other cases of interaction between the drug and the matrix [46-48]. Although, for DPA containing polymers, the total Rh6G released at pH 6.5 (about 90 %) is higher than at pH 8.4 (about 40 %). At acidic pH, the tertiary amine groups are partially protonated and the electrostatic interaction with the Rh6G cation impels the release from the matrix. At pH 8.4, the electrostatic repulsion is no longer acting and, therefore, the driving force is reduced.

Table 3 shows the kinetics parameters (k and n) of the experimental data of Figure 7, calculated using equation 3, and the diffusion coefficient (D), using equation 4.

Table 3. Parameters (k, n) calculated from the fit of Eq. (3), and diffusion coefficients (D_{ip}) calculated from Eq. (4) for the Rh6G release curves from Figure 7

Samples	***pH***	r^2	$k \times 10^2$ (cm^{-1})	***N***	$D_{ip} \times 10^9$ ($cm^2 .seg^{-1}$)
HD100/0-1	6.5	0.996	2.75	0.51	0.82
	7.4	0.986	2.73	0.51	0.94
	8.4	0.998	3.31	0.50	1.23
HD90/10-1	6.5	0.995	2.10	0.67	-
	7.4	0.995	1.58	0.63	-
	8.4	0.993	1.42	0.61	-
HD70/30-1	6.5	0.978	3.17	0.78	-
	7.4	0.993	1.57	0.56	-
	8.4	0.988	1.84	0.48	-

For pure pHEMA samples the n values indicate a Fickian transport from pH 6.5 to 8.4. Highly soluble drugs, like Rh6G, typically exhibit Fickian release from hydrogels, and the release profile is mainly dependent upon the solubility and diffusion kinetics of the drug. For the hydrogel HD90/10-1, the n values are between 0.5 and 1, indicating anomalous transport, and the relaxation process dominates over diffusion. In the case of HD70/30-1, the n values varies significantly with the pH, at pH 6.5 and 7.4 are between 0.5 and 1 while at pH 8.4 is slightly lower than 0.5. For higher pHs the experimental values suggest a different mechanism

406 transport, that is, the presence of another process besides passive diffusion. Above pK_a the
407 deprotonation is accompanied by a de-swelling of the hydrogel (see Figure 2).
408 The D_{ip} value of Rh6G in water at 25 °C is $4.14.10^{-6}$ cm^2/s and as expected, all values found
409 in this work for Fickian diffusion are lower than this one [49].
410 In principle the relation between the pH and the percentage of released R6G indicate that,
411 while the release mechanism can be similar for the different pHs, the final amount of drug
412 released depends on the pH of the medium and on the swelling degree of the material. This
413 behavior demonstrates the ability of such copolymers to achieve a control of drug released as
414 a function of the pH of the medium.
415

416 *3.5.2 Effect of cross-linking density*

417 To evaluate the effect of cross-linker concentration on the mechanism of the drug transport at
418 pH 7.4 and 34.5 °C, the parameters (*n, k*) of the power law model in Eq. (3) are calculated
419 (Table 4).
420

421 Table 4. Parameters (*k, n*) calculated from the fit of Eq. (3) for the Rh6G release from different
422 cross-linking densities at pH 7.4 and 34.5 ºC.

Samples	***Cross-linking (% wt.)***	r^2	$k \times 10^3$ (min^{-1})	***n***
HD100/0	1	0.990	27.28	0.51
	3	0.989	20.72	0.51
HD90/10	1	0.997	15.82	0.61
	3	0.994	10.87	0.59
HD70/30	1	0.996	15.16	0.57
	3	0.980	7.67	0.61

423

424 The release of Rh6G in pHEMA homopolymer at pH 7.4 seems to follow a Fickian diffusion
425 behavior as suggested by the values of *n*. However, by incorporating DPA, the *n* values are
426 close to 0.6 for all contents and cross-linking densities, suggesting a non-Fickian behavior.
427 The mechanism of Rh6G release does not seem to be affected by increasing the cross-linking
428 density for the same copolymer composition, as judged by the *n* values. However, a reduction
429 in the *k* parameter is the most important effect of higher cross-linker concentration. The
430 decrease in kinetic constant values reflects the decrease in the rate of drug release, which
431 might be due to the dominance of chain entanglement and the decrease in the water content of
432 polymers with different cross-linking densities. By increasing the cross-linking density, the

pores are smaller and less water is allowed to enter the matrix. Since Rh6G is a water soluble molecule, this factor has an important impact on the drug releasing rate. The larger pore in the 1 wt. % cross-linked copolymers allows Rh6G diffusion with no or little resistance compared to smaller one. Pore size is significantly impacted by the extent of cross-linking and their increasing values result in lower released kinetic. This behavior could be an advantage in the case of treatments where a prolonged therapy is required.

4. CONCLUSIONS

The incorporation of DPA confers pH-responsive properties to the polymer; copolymers show a significant increase of the swelling degree when the pH decrease below 7.40, and reach the equilibrium around pH 6.0. This effect is directly proportional to the amount of DPA present in the copolymers and inversely proportional with the amount of cross-linker. The apparent pK_a of copolymers depend on the composition of HEMA/DPA and the crosslinking degree of the hydrogels, and the estimated values are between 6.80 and 7.17. SEM images of copolymers show important morphological changes when varying the medium pH according to swelling results. At acid pH, SEM images show an open morphology state with a porous structure as a consequence of the matrix expansion at this pH, while at basic pH show a collapsed state due to lower swelling degree and a more hydrophobic polymer. Hydrogel with 30 %wt of DPA show higher pores in the hydrogels than hydrogels with 10 % wt. For hydrogel HD100/0-1 no changes with pH are appreciated and in all cases a compact surface is observed.

Additionally, the copolymers of HEMA and DPA were tested as drug delivery systems using Rh6G as model drug. The amount of Rh6G incorporated is higher for pure pHEMA than copolymers and depends mainly on the medium pH and the interaction between the drug and the matrix of the copolymers. The FTIR spectra between loaded and unloaded films, indicates that the Rh6G molecules interact with the OH group of the HEMA by hydrogen bonding or through dipole–dipole interaction. At pH 6.5, the total Rh6G uptake is lower than at pH 8.4, and the SEM images show a greater accumulation on the surface at this pH. Thus the loaded is inversely proportional with the swelling of the hydrogel and mainly depends on the interaction between the drug and the matrix of the copolymers. The total release of the drug depends on the polymer composition and medium pH. For pure pHEMA, the drug remains strongly associated with the polymer chains inside the matrix and, therefore, its release is very slow. On the other hand, for copolymers, the total Rh6G released at acid pH is higher than at

basic pH, and it increases as the proportion of DPA monomer increases. The pore size observed from SEM images is highly correlated with the drug release behavior when varying the medium pH. For copolymers, the release of the Rh6G model drug in PBS follows a non-Fickian diffusion process for pHs values less than or equal to 7.4. The change of the polymer´s cross-linking density affects only the drug release rate.

In conclusion, by changing the DPA content and the degree of cross-linking density it is possible to modify the kinetic parameters and, therefore, to control the release kinetics depending on the medium pH. The copolymers of HEMA/DPA are potentially useful as drug delivery systems for ophthalmic therapies.

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REFERENCES

[1] Jagur-Grodzinski J.: Polymeric gels and hydrogels for biomedical and pharmaceutical applications. Polymers for Advanced Technologies, **21**, 27-47 (2010). DOI: 10.1002/pat.1504.

[2] Alvarez-Lorenzo C., Concheiro A.: Molecularly imprinted materials as advanced excipients for drug delivery systems. Biotechnol Annu Rev, **12**, 225-68 (2006). DOI: 10.1016/s1387-2656(06)12007-4.

[3] Geever L. M., Cooney C. C., Lyons J. G., Kennedy J. E., Nugent M. J., Devery S., Higginbotham C. L.: Characterisation and controlled drug release from novel drug-loaded hydrogels. Eur J Pharm Biopharm, **69**, 1147-59 Aug (2008). DOI: 10.1016/j.ejpb.2007.12.021.

[4] García D. M., Escobar J. L., Noa Y., Bada N., Hernáez E., Katime I.: Timolol maleate release from pH-sensible poly(2-hydroxyethyl methacrylate-co-methacrylic acid) hydrogels. European Polymer Journal, **40**, 1683-1690 8// (2004). DOI: 10.1016/j.eurpolymj.2004.03.012.

[5] Bettini R., Colombo P. and Peppas N.A.: Solubility effects on drug transport through pH-sensitive, swelling-controlled release systems: Transport of theophylline and metoclopramide monohydrochloride. Journal of Controlled Release, **37**, 105-111 (1995).

[6] Atta A. M., Arndt K. F.: Swelling behavior of pH- and temperature-sensitive copolymers containing 2-hydroxy-ethyl methacrylate and N-vinyl-2-pyrrolidone crosslinked with new crosslinkers. Polymer International, **53**, 1870-1881 (2004). DOI: 10.1002/pi.1606.

[7] Peppas N. A., Khare A. R.: Preparation, structure and diffusional behavior of hydrogels in controlled release. Advanced Drug Delivery Reviews, **11**, 1-35 7// (1993).

508 DOI: 10.1016/0169-409X(93)90025-Y.
509 [8] Xinming L., Yingde C., Lloyd A. W., Mikhalovsky S. V., Sandeman S. R., Howel C.
510 A., Liewen L.: Polymeric hydrogels for novel contact lens-based ophthalmic drug
511 delivery systems: A review. Contact Lens and Anterior Eye, **31**, 57-64 4// (2008).
512 DOI: 10.1016/j.clae.2007.09.002.
513 [9] Richter A., Paschew G., Klatt S., Lienig J., Arndt K.-F., Adler H.-J.: Review on
514 Hydrogel-based pH Sensors and Microsensors. Sensors, **8**, 561-581 (2008).
515 [10] Casolaro M., Casolaro I., Lamponi S.: Stimuli-responsive hydrogels for controlled
516 pilocarpine ocular delivery. Eur J Pharm Biopharm, **80**, 553-61 Apr (2012).
517 DOI: 10.1016/j.ejpb.2011.11.013.
518 [11] Alvarez-Lorenzo C., Hiratani H., Concheiro A.: Contact lenses for drug delivery.
519 American Journal of Drug Delivery, **4**, 131-151 2006/09/01 (2006).
520 DOI: 10.2165/00137696-200604030-00002.
521 [12] Urtti A., Salminen L.: Minimizing systemic absorption of topically administered
522 ophthalmic drugs. Survey of Ophthalmology, **37**, 435-456 5// (1993).
523 DOI: 10.1016/0039-6257(93)90141-S.
524 [13] del Amo E. M., Urtti A.: Current and future ophthalmic drug delivery systems: A shift
525 to the posterior segment. Drug Discovery Today, **13**, 135-143 2// (2008).
526 DOI: 10.1016/j.drudis.2007.11.002.
527 [14] Lin C. C., Metters A. T.: Hydrogels in controlled release formulations: network design
528 and mathematical modeling. Advanced Drug Delivery Reviews, **58**, 1379-408 Nov 30
529 (2006).
530 DOI: 10.1016/j.addr.2006.09.004.
531 [15] Evans D. F., Pye G., Bramley R., Clark A. G., Dyson T. J., Hardcastle J. D.:
532 Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut,
533 **29**, 1035-41 Aug (1988).
534 [16] Carney L. G., Hill R. M.: Human tear pH. Diurnal variations. Arch Ophthalmol, **94**,
535 821-4 May (1976).
536 [17] Baeyens V., Gurny R.: Chemical and physical parameters of tears relevant for the
537 design of ocular drug delivery formulations. Pharm Acta Helv, **72**, 191-202 Sep
538 (1997).
539 [18] Jaros P. A., Coles W. H.: Ocular surface pH in Rosacea. CLAO J, **9**, 333-6 Oct-Dec
540 (1983).
541 [19] Norn M. S.: Tear fluid pH in normals, contact lens wearers, and pathological cases.
542 Acta Ophthalmologica, **66**, 485-9 Oct (1988).
543 [20] Bawa P., Pillay V., Choonara Y. E., du Toit L. C.: Stimuli-responsive polymers and
544 their applications in drug delivery. Biomed Mater, **4**, 022001 Apr (2009).
545 DOI: 10.1088/1748-6041/4/2/022001.
546 [21] París R., Quijada-Garrido I.: Temperature- and pH-responsive behaviour of poly(2-(2-
547 methoxyethoxy)ethyl methacrylate-co-N,N-dimethylaminoethyl methacrylate)
548 hydrogels. European Polymer Journal, **46**, 2156-2163 11// (2010).
549 DOI: 10.1016/j.eurpolymj.2010.09.004.
550 [22] Emileh A., Vasheghani-Farahani E., Imani M.: Swelling behavior, mechanical
551 properties and network parameters of pH- and temperature-sensitive hydrogels of
552 poly((2-dimethyl amino) ethyl methacrylate-co-butyl methacrylate). European
553 Polymer Journal, **43**, 1986-1995 5// (2007).
554 DOI: 10.1016/j.eurpolymj.2007.02.002.
555 [23] Andrade-Vivero P., Fernandez-Gabriel E., Alvarez-Lorenzo C., Concheiro A.:
556 Improving the loading and release of NSAIDs from pHEMA hydrogels by
557 copolymerization with functionalized monomers. J Pharm Sci, **96**, 802-13 Apr (2007).

DOI: 10.1002/jps.20761.
[24] Amalvy J. I., Wanless E. J., Li Y., Michailidou V., Armes S. P., Duccini Y.: Synthesis and characterization of novel pH-sensitive microgels based on tertiary amine methacrylates. Langmuir, **21**, 8992-8999 (2004).
[25] Faccia P. A., Amalvy J. I.: Synthesis, characterization, and swelling behavior of new pH-sensitive hydrogels derived from copolymers of 2-hydroxyethyl methacrylate and 2-(diisopropylamino)ethylmethacrylate. Journal of Applied Polymer Science, **127**, 1974-1980 (2013).
[26] Firestone B. A., Dickason M. A., Tran T.: Solubility characteristics of three fluoroquinolone ophthalmic solutions in an in vitro tear model. International Journal of Pharmaceutics, **164**, 119-128 // (1998).
DOI: 10.1016/S0378-5173(97)00427-4.
[27] Milder B.: The lacrimal apparatus, in Adler's Physiology of the eye: clinical application, St. Louis, Mosby (1987).
[28] De Jalón E. G., Blanco-Príeto M. J., Ygartua P., Santoyo S.: PLGA microparticles: Possible vehicles for topical drug delivery. International Journal of Pharmaceutics, **226**, 181-184 (2001).
[29] Ritger P. L., Peppas N. A.: A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. Journal of Controlled Release, **5**, 37-42 (1987).
[30] Ritger P. L., Peppas N. A.: A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. Journal of Controlled Release, **5**, 23-36 6// (1987).
DOI: doi.org/10.1016/0168-3659(87)90034-4.
[31] Bajpai A. K., Shukla S. K., Bhanu S., Kankane S.: Responsive polymers in controlled drug delivery. Progress in Polymer Science, **33**, 1088-1118 11// (2008).
DOI: 10.1016/j.progpolymsci.2008.07.005.
[32] Karlgard C. C. S., Wong N. S., Jones L. W., Moresoli C.: In vitro uptake and release studies of ocular pharmaceutical agents by silicon-containing and p-HEMA hydrogel contact lens materials. International Journal of Pharmaceutics, **257**, 141-151 5/12/ (2003).
DOI: 10.1016/S0378-5173(03)00124-8.
[33] Brannon-Peppas L., Peppas N. A.: Equilibrium swelling behavior of pH-sensitive hydrogels. Chemical Engineering Science, **46**, 715-722 (1991).
[34] Chirila T. V., Chen Y.-C., Griffin B. J., Constable I. J.: Hydrophilic sponges based on 2-hydroxyethyl methacrylate. I. effect of monomer mixture composition on the pore size. Polymer International, **32**, 221-232 (1993).
DOI: 10.1002/pi.4990320303.
[35] Nam K., Watanabe J., Ishihara K.: Modeling of swelling and drug release behavior of spontaneously forming hydrogels composed of phospholipid polymers. International Journal of Pharmaceutics, **275**, 259-69 May 4 (2004).
DOI: 10.1016/j.ijpharm.2004.02.009.
[36] Pinzón N., Espinosa A., Perilla J., Hernáez E., Katime I.: Modelamiento del hinchamiento y difusión de solutos en hidrogeles. Revista Iberoamericana de Polímeros, **3**, 38-54 (2002).
[37] Lowman A. M., Peppas N. A.: Encyclopedia of controlled drug delivery Vol 1. John Wiley & Sons, Inc., Brown University, Providence, Rhode Island (1999).
[38] Chen J., Park H., Park K.: Synthesis of superporous hydrogels: hydrogels with fast swelling and superabsorbent properties. J Biomed Mater Res, **44**, 53-62 Jan (1999).

[39] Chen J. U. N., Park K.: Superporous hydrogels: Fast responsive hydrogel systems. Journal of Macromolecular Science, Part A, **36**, 917-930 1999/01/01 (1999). DOI: 10.1080/10601329908951189.
[40] Tleugabulova D., Sui J., Ayers P. W., Brennan J. D.: Evidence for Rigid Binding of Rhodamine 6G to Silica Surfaces in Aqueous Solution Based on Fluorescence Anisotropy Decay Analysis. The Journal of Physical Chemistry B, **109**, 7850-7858 2005/04/01 (2005). DOI: 10.1021/jp045569r.
[41] Jin L., Deng Y., Hu J., Wang C.: Preparation and characterization of core–shell polymer particles with protonizable shells prepared by oxyanionic polymerization. Journal of Polymer Science Part A: Polymer Chemistry, **42**, 6081-6088 (2004). DOI: 10.1002/pola.20453.
[42] Watanabe H., Hayazawa N., Inouye Y., Kawata S.: DFT vibrational calculations of rhodamine 6G adsorbed on silver: analysis of tip-enhanced Raman spectroscopy. Journal of Physical Chemistry B, **109**, 5012-5020 Mar 24 (2005). DOI: 10.1021/jp045771u.
[43] Tripathi S. K., Monga A., Saini G. S. S.: Characterization of thermally evaporated thin films of Rhodamine 6G. Smart Materials and Structures, **18**, (2009). DOI: 10.1088/0964-1726/18/12/125012.
[44] Saini G. S., Kaur S., Tripathi S. K., Mahajan C. G., Thanga H. H., Verma A. L.: Spectroscopic studies of rhodamine 6G dispersed in polymethylcyanoacrylate. Spectrochim Acta A Mol Biomol Spectrosc, **61**, 653-8 Feb (2005). DOI: 10.1016/j.saa.2004.05.022.
[45] Tomić S. L., Mićić M. M., Filipović J. M., Suljovrujić E. H.: Swelling and drug release behavior of poly(2-hydroxyethyl methacrylate/itaconic acid) copolymeric hydrogels obtained by gamma irradiation. Radiation Physics and Chemistry, **76**, 801-810 5// (2007). DOI: 10.1016/j.radphyschem.2006.05.013.
[46] Momose T., Ito N., Kanai A., Watanabe Y., Shibata M.: Adsorption of levocabastine eye drops by soft contact lenses and its effects in rabbit eyes. CLAO J, **23**, 96-9 Apr (1997).
[47] Lumbroso P., Nhamias M., Nhamias S., Tranche P.: A preliminary study of the adsorption and release of preservatives by contact lenses and collagen shields. CLAO J, **22**, 61-3 Jan (1996).
[48] Miranda M. N., Garcia-Castineiras S.: Effects of pH and some common topical ophthalmic medications on the contact lens Permalens. CLAO J, **9**, 43-8 Jan-Mar (1983).
[49] Gendron P. O., Avaltroni F., Wilkinson K. J.: Diffusion coefficients of several rhodamine derivatives as determined by pulsed field gradient-nuclear magnetic resonance and fluorescence correlation spectroscopy. J Fluoresc, **18**, 1093-101 Nov (2008). DOI: 10.1007/s10895-008-0357-7.

Legends of figures and tables

Figures

Figure 1: Chemical structure of Rhodamine 6G cation

Figure 2: Equilibrium swelling degree for copolymers as a function of pH at 34.5 °C.

Tables